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Amnesic Effects of Lithium Chloride in Chicks

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Newly-hatched chicks were given intracranial injections of lithium chloride either before or after being trained in a one-trial passive avoidance task. Twenty minutes after training, memory of the aversive experince was found to be unaffected regardless of the time injections had been given. Retrieval measured 24 hr later, however, was severely impaired in chicks that had been injected 4 min before training, less so in those injected 2 min before, and unaffected in chicks treated 10 sec after training. Thus, while LiCl does not interfere with mnemonic processes necessary for short-term retrieval, it does seem to act with a delayed effectiveness to disrupt an early stage in the development of long-term memory. Previous studies indicate that immediately upon training a metastable memory trace becomes activated which seems to function as behaviorally-retrievable memory for the next hour or so, and which, within the first 45 sec, induces the growth of a behaviorally latent precursor to long-term memory. The present effects of LiCl can be interpreted in these terms as having selectively disrupted formation of the latter precursor component.

INTRODUCTION

The amnesic effects of various agents administered before or after a training experience have been used to investigate the physiological bases of memory formation (e.g., 13, 14). A series of such studies utilizing a one-trial aversive conditioning paradigm in chicks begun in this laboratory about eight years ago and since continued here and elsewhere (3–6, 8–12) has led to the tentative identification of a sequence of events involved in the formation of an engram. These studies indicate that within 45 sec after training a relatively stable precursor to long-term memory is formed (10). The growth of this memory trace seems to be induced by a metastable

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process that is activated within a fraction of a second after the aversive experience and which then persists at a constant intensity (4). Over a period of an hour or more, the precursor component, which is not itself behaviorally accessible, apparently gives rise to the permanent engram (5, 6, 9, 11). During this same period, short-term retrieval seems to be made possible by means of a distinct, electroshock-sensitive form of memory (9) that has been postulated (4) to represent the continued activity of the initial metastable trace. A similar model, proposed by McGaugh and Dawson (14), has also emerged from a large number of experiments in mammals.

It has recently been shown by Mark and Watts (11) that LiCl disrupts memory for the one-trial task in chicks if administered five minutes before, but not ten minutes after, training. Since LiCl also caused a somewhat accelerated decline in chicks' short-term memory, these authors hypothesiezd that the amnesic effects of the drug are due to an interference with a short-term memory trace, the duration or intensity of which determines the amount of long-term memory (12). Studies using electroshock have shown, however, that the critical events linking short- and long-term memory are completed within the first minute (8–10), at which time no significant short-term retrieval deficits had yet appeared in the study of Mark and Watts (12). In terms of the model proposed above, the amnesic effects of LiCl could alternatively be interpreted as an interference with the formation of the long-term memory precursor in the first 45 sec. To determine whether this might be the case, the present study examined the time course of the action of LiCl in greater detail, while also re-examining the effects of the drugs on chicks' short-term memory.

MATERIALS AND METHODS

White Leghorn cockerels were obtained from a commercial hatchery the day after hatching. Chicks were housed in individual cartons throughout the experiment. The experimental room was maintained at 31.1 C and 40% humidity; lights were on between 6:30 AM and 6:30 PM. No food or water were provided, since adequate nutrients are available from the yolk sac for the first few days after hatching.

The task on which chicks were trained involved learning to suppress their innate pecking tendency on the basis of a single aversive experience (10). Prior to conditioning, about 90% of naive chicks will peck at a small shiny metallic bead within 5 sec of its presentation. When this object has been coated with methyl anthranilate (MeA), chicks typically shake their heads in a "disgust" reaction upon tasting the aversant. In the first 30 min after training, chicks have an 80–85% probability of avoiding

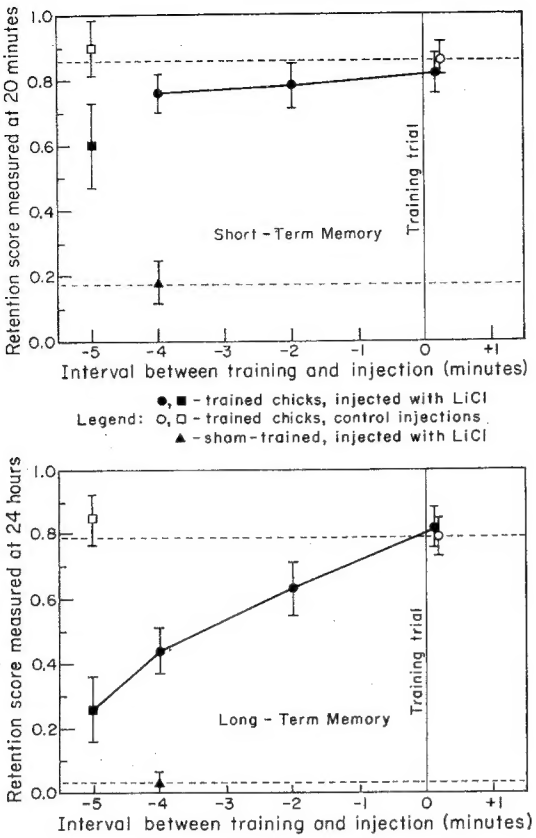

FIG. 1. Retrieval 20 min after training (top) and 24 hr after training (bottom), plotted as functions of the interval between the training trial and the injection of lithium chloride. In each of these figures, the top dotted line represents the fraction of trained controls avoiding the lure on the retention test, while the bottom line represents the indigenous aversive reaction of untrained controls treated with LiCl. Squares represent data of Mark and Watts (in the top figure, the dosage of LiCl used by those authors was 33% higher than that used here). Bars indicate mean errors.

the aversive stimulus upon re-presentation (9) and about a 75% probability of avoiding it a day later (10).

To examine the changes in effectiveness of LiCl in disrupting memory processes, the drug was administered either 4 min ($N = 45$) or 2 min ($N = 38$) prior to the training trial, or 10 sec afterwards ($N = 44$) (i.e., injection procedure was completed 10 sec after training). Bilateral intracranial injections were placed 3 mm beneath the dura, in the region of the hyperstriatum venrale. Each chick was given 245 μg LiCl in 0.05 ml of

chick Ringer's solution, divided into two equal portions for injection into the two cerebral hemispheres. Mark and Watts have previously established this dosage as severely impairing long-term memory formation while not affecting gross changes in alertness, posture, locomotion, or pecking ability. In about half of the population, injection sites had been marked in advance. The injection procedure lasted about 7 sec per chick.

To determine whether the injection *per se* influence learning, one group of controls (C_2; $N = 44$) was given Ringer's solution within 10 sec of training. Another group of controls (C_1; $N = 32$) was given LiCl injections and then sham-trained 4 min later using a lure not coated with MeA in order to establish that the short-term behavioral impairments caused by LiCl injections were indeed related to memory deficits.

Retention was measured in all chicks at both 20 min and 24 hr after training. Chicks were pseudorandomly mixed together, and testing was essentially blind. At each of the testing intervals, chicks were given one presentation of a lure resembling that used in training but not coated with MeA. The scores shown in Fig. 1 represent the fraction of chicks in a group that avoided the lure on each retention trial.

RESULTS

As shown in Fig. 1 (top), LiCl given either before or after training did not impair retrieval 20 min after the aversive experience. Avoidance in the control group injected with Ringer's solution 10 sec after training (Group C_1) was similar to the average value of 0.85 obtained by Lee-Teng, Magnus, Kanner, and Hochman (9) 1–30 min after training in chicks given no treatment at all. Sham-trained controls (C_2) showed some loss of indigenous pecking as a result of the injections. However, the aversive reaction in chicks given LiCl 4 min before training was substantially greater than that of these untrained controls ($\chi^2 = 25.8$, $P < 0.001$) and not significantly different from that of the fully-trained controls (i.e., Group C_1) ($\chi^2 = 1.68$, $P \sim 0.20$). The other two trained, LiCl-injected groups resembled group C_1 even more closely (Fig. 1, top).

In contrast to the unimpaired retrieval seen 20 min after training, chicks that had been given LiCl 4 min before training showed a severe retention deficit when tested 24 hr later. Long-term memory in the various groups is shown in the bottom of Fig. 1. The fraction of chicks avoiding the lure in the Ringer's-injected, trained controls (C_1) resembles the value found previously in trained chicks given no injections (10). Previous controls from the studies of Mark and Watts (11, 12), also shown in Fig. 1, bottom, demonstrate that injections of saline 5 min before training have no effect on chicks' long-term memory. Retention in chicks given LiCl 4

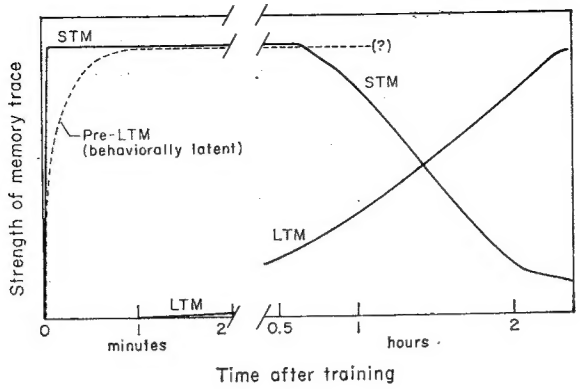

FIG. 2. Schematic representation of the mnemonic processes that result from one-trial aversive conditioning in the chick. Short-term memory (STM), which is susceptible to disruption by electroshock, becomes activated immediately upon training (4). Retrograde amnesia studies using electroshock indicate that within 45 sec this induces the growth of the electroshock-insensitive Pre-LTM trace (4, 8–10), a process suggested here to be susceptible to disruption by LiCl. Pre-LTM is not behaviorally retrievable (9), but subsequently gives rise to a permanent engram. During this transitional period, the initial induction process seems to continue to serve as a behaviorally retrievable memory trace. In the presence of cycloheximide, which presumably blocks LTM synthesis, the decay of the STM trace can be observed independently (12).

min before training differed significantly from that of Group C_1 ($\chi^2 = 11.1$, $P < 0.001$). Chicks given LiCl 2 min before training showed a less severe deficit, and their score did not differ from that of the controls at a conventional level of significance ($\chi^2 = 2.51$, $P \sim 0.10$). Retention in chicks given LiCl 10 sec after training closely resembled that of controls: $\chi^2 = 0.10$, $P > 0.70$. Untrained controls (C_2) avoided the lure only 3% of the time.

DISCUSSION

Experiments by Mark and Watts (11) showed that LiCl severely impairs chicks' long-term memory if injected 5 min before, but not 10 min after, the training trial. For injections given between these two intervals, the present results indicate that the drug becomes increasingly less effective in disrupting long-term memory as injections are given closer to the time of training, and the drug is completely ineffective if administered even 10 sec afterwards. On the other hand, retrieval of the aversive experience 20 min after training was unaffected by injections given either before or after training. It would therefore seem that the first 20 minutes or more of short-term memory are not dependent upon mnemonic processes affected

by LiCl; however, the drug does act with delayed effectiveness to interfere with some early stage in the development of chicks' long-term memory.

Studies using electroshock to disrupt memory consolidation in chicks indicate that in the first 45 seconds after training, a relatively stable precursor to long-term memory becomes formed (10). The growth of this trace seems to be induced by a metastable process that is activated immediately upon training and which then persists at a constant intensity (4). The long-term memory precursor, which itself seems not to be accessible for behavioral expression, apparently gives rise to the permanent engram in the next hour or more (6, 9, 11). During this same period, short-term retrieval seems to be made possible by means of a distinct, electroshock-sensitive memory trace (9) postulated to represent a continuation of the initial metastable induction process (4) (see Fig. 2). In terms of this model, the memory deficits found here suggest that when administered a sufficiently long time before training, the slow acting LiCl interferes with formation of the long-term memory precursor.

Increasingly larger doses of LiCl also seem to accelerate the decline of short-term memory to some degree (12). However, this effect is not yet apparent for the first few minutes after training (12), at which time the sequence of events linking short- and long-term memory have already been completed (9, 10). Therefore, the long-term amnesic effects of LiCl can not be attributed to the action of the drug on short-term memory, as suggested by Mark and Watts (11, 12), but rather to a specific blockage of a distinct long-term memory precursor. In conformity with this hypothesis, the somewhat anomalous finding (12) that low doses of LiCl yield memory scores at 24 hr higher than those observed at 30, 60, or 90 min can be explained as the injections having caused a somewhat accelerated decline in short-term memory while having allowed enough of the long-term memory precursor to be formed in the first minute to give rise to the memory seen the next day.

Lithium ions apparently pass inward through neuronal membranes and allow action potential to be transmitted normally, but, once inside nerve cells, they are not actively extruded by the "sodium pump" (7). It is thus conceivable that the earliest stages of memory that are unaffected by LiCl might involve some pattern of neural excitation, while the subsequent storage of the long-term memory precursor could correspond to more stable neuronal membrane changes induced by such excitation. The post-tetanic hyperpolarization that results from repeated stimulation of neuorns and which has been shown to be blocked by LiCl (15) has been suggested as one possible candidate for the latter type of memory storage (11, 12). It seems difficult to believe, however, that an ongoing electrical pattern could be maintained for the length of time STM has been shown to persist

(e.g., 1, 2, 12), and it would therefore seem that the STM trace might be stored by means of a different metastable process that is affected by electroshock but not by LiCl.

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